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Abstract

Purpose

Identification of biomarkers in Major Depressive Disorder (MDD) has proceeded in an extemporized manner. No single biomarker has been identified with utility in screening, diagnosis, prognosis or monitoring, and screening tests have different characteristics than the other functions. Using Chaos, Bifurcation and Perturbation (CBP) Theories, the aim is to identify biomarkers to aid clinicians in screening for MDD.

Materials and methods

MDD is a complex disorder; consequently, a reductionist approach to characterize the complex system changes found in MDD will be inchoate and unreliable. A holistic approach is used to identify biomarkers reflecting the tipping points seen before the catastrophic bifurcation that results in MDD.

Results

Applying CBP Theories revealed skew, resistance to change, flickering, increased variance and autocorrelation as patterns of biomarkers. Integrals and differentials of extracellular and intracellular biomarkers were identified, specifically focussed on hypothalamo-pituitary axis (HPA) axis dysfunction, metabolic dysfunction, inflammation and mitochondrial oxidative stress, and tryptophan metabolism.

Conclusion

Applying CBP Theories to the dysfunctional complex biological systems in MDD led to development of integrals and differentials of biomarkers that can be used in screening for MDD and planning future biomarker research, targeting intracellular and extracellular inflammation, HPA axis dysfunction, and tryptophan metabolism.

Key Words: Bifurcation Theory, Chaos Theory, biomarkers, depression, major depressive disorder, depression screening, Perturbation Theory.

Introduction

Major Depressive Disorder (MDD) causes significant functional impairment (Kessler *et al.*, 2003), incurs significant public health costs (Kessler, 2012), and contributes to approximately 10% of the global burden of disease (World Health Organisation, 2009). Moreover, there is evidence of an increase in the rate of MDD (Ferrari *et al.*, 2012). The US Preventive Services Task Force has recommended that primary care providers should screen all adults for MDD (Siu *et al.*, 2016).

Biomarker research has focused on the development of a single biomarker or small group of biomarkers that can be used to diagnose, rather than screen for, MDD (Boksa, 2013, Ebot Enaw and Smith, 2013, Bilello *et al.*, 2015, Kunugi *et al.*, 2015, De Long, 2016, Young *et al.*, 2016). Commentary in the American Association of Clinical Chemistry (De Long, 2016) and others (Kunugi *et al.*, 2015) suggest that the reductionist approach of searching for a single test for MDD has been unrewarding. Twelve recent reviews from 2011 to 2017, including those above, identified different arrays of biomarkers with only minimal overlap in core biomarkers between reviews (Papakostas *et al.*, 2011, Lang and Borgwardt, 2013, Lopresti *et al.*, 2014, Seib *et al.*, 2014, Zheng *et al.*, 2016, Harada *et al.*, 2017, Machado-Vieira *et al.*, 2017, Strawbridge *et al.*, 2017). Recent advances have considered a group of tests, forming a profile of biomarkers such as the MDDScore, rather than a single test (Bilello *et al.*, 2015).

MDD is a complex biological disorder where multiple biological systems have been shown to be dysfunctional (Stapelberg *et al.*, 2015). It is proposed that a strategic

and holistic approach to the development of biomarkers for MDD is required. In addition, differing biomarkers for screening, diagnosis, monitoring and prognosis in MDD are required because the characteristics of tests required fulfilling these functions are significantly different.

Tipping points occur before catastrophic bifurcations (Scheffer *et al.*, 2009) such as the transition from health to MDD and involve dysfunction of the HPA axis (Sapolsky, 2000), metabolic state (Winokur *et al.*, 1988), tryptophan metabolism (Ruddick *et al.*, 2006), inflammatory control and mitochondrial oxidative stress (Khanzode *et al.*, 2003). Applying Chaos, Bifurcation and Perturbation Theories to these dysfunctional biological systems facilitates development of a profile of biomarkers to screen high prevalence groups for MDD. As the systems and the changes within them are so complex, it is proposed that biomarkers which are measured as absolute levels, integrals and differentials will show the skew, resistance to change, flickering, increased variance, and autocorrelation seen in tipping points prior to catastrophic bifurcation, and will be significantly more valuable than a single biomarker measured at a single point in time.

Biomarkers in MDD

MDD is currently defined using a categorical classification system. DSM 5 (American Psychiatric Association, 2013) and ICD 10 (World Health Organization, 1992), both delineate diagnostic criteria for MDD that are based on subjective patient assessments and structured, but subjective, clinician assessments. There have been attempts to introduce objective testing to aid in diagnosis, screening, prognosis, or monitoring of treatment response for psychiatric disorders. For example, the inclusion of cerebrospinal fluid hypocretin-1 (orexin-A) testing into the diagnostic criteria for

narcolepsy represents the first use of a laboratory test to support a formal psychiatric diagnosis (criterion (B2), p373 in DSM 5 (American Psychiatric Association, 2013)).

An alternative approach has been to use multiple biomarkers together as a single investigative entity (Papakostas *et al.*, 2011), such as the MDDScore test (Bilello *et al.*, 2015). Sensitivity and specificity of the MDDScore test have been reported to be as high as 92% and 81% respectively.

Using an holistic approach, the intent in this paper is to identify from the literature by applying the methodology delineated, an initial group of analytes that could be used as biomarkers of the tipping points that are precursors to the clinical depressive state.

Clinical Significance

- Strategic methodology for the development of biomarkers in MDD
- Developing biomarkers for aiding in screening high risk populations for MDD
- Developing biomarkers for aiding in diagnosis of MDD
- Developing prognostic biomarkers for MDD

Materials and Methods

Functions of Tests or Biomarkers

Tests of individual biomarkers are undertaken for one of four main reasons (Mayeux, 2004, Pariante and Lightman, 2008a, Burtis *et al.*, 2015a):

- Screening
- Diagnosis, including identification of subtypes
- Monitoring disorder progress or treatment response
- Prognosis

As the characteristics of each are inherently different, this initial research is restricted to screening for MDD targeting the tipping point transformations that occur prior to catastrophic bifurcations.

Application of foundation theories

Perturbation Theory is a method for finding an approximate solution to a complex problem by starting from the exact solution of a related, simpler problem. A critical feature of the technique is a middle step that breaks the problem into "solvable" and "perturbation" parts (Bogolyubov, 2011). Perturbation Theory was applied to the problem of developing biomarkers in MDD. In a myocardial infarction, changes in a single test such as troponin I can be used in initial screening of high risk patients with chest pain, and, after a repeat test, diagnose myocardial infarction. The change over time is the diagnostic criterion. From this solvable part, the concept of interpolating the variable of time into biomarker measurement in MDD gave rise to the perturbations suggesting value in integrals and differentials of biomarker measurements in the complex disorder that is MDD.

Chaos Theory describes dynamical systems that evolve over time. These complex systems are highly sensitive to initial conditions in a deterministic way, but can have major perturbations over the long term, which, as described by the butterfly effect, can lead to catastrophic bifurcations and result in new stable pathological states (Ott, 2002, Boeing, 2016). Feedback often becomes chaotic prior to critical transition to the new stable, but pathological, state. The state prior to the catastrophic bifurcation is a tipping point and may herald development of the new pathological state. Identifying tipping points could serve as potential indicators for the development of MDD.

Bifurcation Theory is most commonly applied to the study of dynamical systems and is concerned with the sudden changes that occur in a system when one or more parameters are varied (Arnold and Novikov, 1994). When combined with Chaos Theory, it is proposed that, as MDD is a complex biological system disorder and that a reductionist approach has not led to any definitive screening or diagnostic biomarkers for MDD, integrals and differentials of profiles of biomarkers can generate appropriate screening biomarkers for MDD.

MDD causes disruption to the Psycho-immuno-neuro-endocrine (PINE) network with dysfunction in immune, autonomic, metabolic and endocrine functionality (Stapelberg *et al.*, 2015). Therefore, MDD may be characterized by a disruption of multiple biomarkers, which reflect multiple changes in the complex pathophysiological system underlying MDD. The effect of MDD on various biological systems has been the focus of research for several decades but the search for a single objective test has been fruitless (Kunugi *et al.*, 2015) so it is proposed that a profile of biomarkers reflecting changes in absolute levels of biomarkers, in responsiveness and reactivity of systems, and/or changes in regulatory set points around which biomarkers vary pathophysiologically (Juster *et al.*, 2010) would have greater clinical utility.

A Mathematical Hierarchy of Predictive Biomarkers

Scheffer et al (Scheffer *et al.*, 2009) proposed a generic categorical classification for early warning systems for tipping points before they reach catastrophic bifurcations. Changes in biomarkers within the complex systems that are dysfunctional in MDD can be summarized into five categories as categories of changes seen prior to catastrophic bifurcations:

- *skew*
- slowing response or *resistance* to change
- *flickering*
- increased *variance*
- *autocorrelation*.

Additionally, applying Perturbation Theory for developing a methodological framework into biomarker research in MDD, these categories can be simplified into first, second and third derivative differentials and integrals of biomarkers with respect to time. The starting point is the initial absolute level of biomarkers at a single point in time, generating one of the five types of tipping point changes proposed by Scheffer et al, and can be expressed mathematically as:

The first derivative differential of the rate of change in biomarker concentration is *skew* (s) where

$$s = d[b]/dt$$

reflects change in the balance of active products or end products within the complex system. For example tryptophan, kynurenine, quinolinic acid and serotonin, where the absolute level or a ratio of absolute levels are the relevant biomarkers. Similarly, skew is reflected in an increase in antithyroperoxidase antibodies (anti-TPO) and anti-thryotropin (anti-TSH) receptor antibodies from baseline over time and are the first derivative differentials.

- The second derivative differential of the rate of change in biomarker concentration is slowing response or *resistance* to change (r) where

$$r = d^2[b]/dt^2 = ds/dt$$

reflects change in the regulatory sensitivity of feedback loops and control within a complex system, for example, changes to cortisol sensitivity with loss of dexamethasone suppression or elevated early morning/awakening cortisol.

- The third derivative differential of the rate of change in biomarker concentration is *flickering* (k), where

$$k = d^3[b]/dt^3 = dr/dt$$

reflects change in heart rate variability (HRV) and is linked with MDD (Kemp *et al.*, 2010).

- V*ariance* (σ) is defined by the area under the curve, or definite integral, of the biomarker concentration [b] as a function of time, where

$$\sigma = \int f[b]\, dt$$

reflects changes in the operating set points of a complex system and its feedback loops. For example, 24-hour urine metanephrine is the definite integral of catecholamine production over time and HbA1c is the definite integral of plasma glucose over 3 months with approximately half related to the previous month and the remaining half resulting from elevated glucose levels in the 2nd and 3rd months of the red cell's life (Burtis *et al.*, 2015b).

- The Laplace transformation of the biomarker concentration is *autocorrelation* (a) and is seen as repeating patterns of flickering or change in variance. It can be positive or negative where there is increasing or reducing amplitude of episodes of flickering or variance respectively. For screening purposes, positive autocorrelation indicates that resistance to change has occurred and the biological system under analysis has changed from a negative feedback inhibition controlled system to one showing impaired control and markedly changed response to stimuli. It reflects moving-average model from changes in sensitivity of feedback loops resulting in repeating patterns of altered responses

to changes over time and here ρ is the frequency of changes in biomarker concentration [b]:

$$a = \int f(t)e^{-\rho t}\,dt$$

For example, cortisol shows episodic release, dynamic responsiveness to environmental and internal stimuli as well as a diurnal rhythm. The Trier Social Stress Test (TSST) shows responses to stress and recovery from it. Repeated TSSTs would show autocorrelation, system responsiveness and dysfunctional system reactivity and recovery (Kirschbaum *et al.*, 2008, Campbell and Ehlert, 2012). Similarly, repeated HbA1c estimations would reveal autocorrelation or recurring patterns of increased variance and change in the moving average. Again, $5HT_{1A}$ receptors are up- and down-regulated in response to rates of change in serotonin activity (Zhang *et al.*, 2014, Andrews *et al.*, 2015). So, measuring receptor activity over time reveals autocorrelation as the end response to acceleration and deceleration of the rate of change of serotonin activity on cells.

Results

Proposed Biomarkers in MDD

Biomarkers were identified from reviews of MDD from 2010 where there was evidence of dysfunction in depression (Papakostas *et al.*, 2011, Boksa, 2013, Ebot Enaw and Smith, 2013, Lang and Borgwardt, 2013, Lopresti *et al.*, 2014, Seib *et al.*, 2014, Bilello *et al.*, 2015, Kunugi *et al.*, 2015, Young *et al.*, 2016, Zheng *et al.*, 2016, Harada *et al.*, 2017, Machado-Vieira *et al.*, 2017, Strawbridge *et al.*, 2017). The mathematical models were applied to the biomarkers identified and, if a biomarker had the potential to reflect one of the five patterns seen before a catastrophic bifurcation, it was included in

the model as a proposed tipping point biomarker. All other biomarkers were excluded, specifically, those that did not have the potential to reflect one of the five patterns occurring before catastrophic bifurcations or those that did not reflect changes in the two extracellular and two intracellular systems identified. Allocation of biomarkers to the intracellular and extracellular subgroups was arbitrary because many of the biomarkers are involved in feedback loops in more than one subgroup. For example, tumour necrosis factor alpha (TNFα) is involved in activation of the HPA axis, stimulates indole dioxygenase in tryptophan metabolism, and activates pro-inflammatory cytokines as well as the serotonin (5-HT) transporter. Biomarkers were grouped extracellularly or intracellularly and were further subdivided into the two most relevant domains that are dysfunctional in MDD:

- Extracellularly, HPA axis dysfunction and metabolic dysfunction
- Intracellularly, altered tryptophan/kynurenine/serotonin metabolism, and inflammation and mitochondrial oxidative stress.

The list of proposed biomarkers is not exhaustive and is given in Tables 1 and 2.

Evidence supporting biomarker utility in screening for MDD

Extracellular Tipping Point Biomarkers

A. HPA Dysfunction and Glucocorticoid Resistance

Salivary Cortisol

Dysfunctional activity of the HPA axis with chronic stress is derived from its harmful effects on dendritic processes, impaired neurogenesis, reduced neuroplasticity and neuronal loss (Sapolsky, 2000). There is no standardised guidance on measurement and use of salivary cortisol (Ryan *et al.*, 2016) but there has been a user's guide developed to assist researchers and practitioners (Hayes *et al.*, 2016), which may help reduce

variability of results found in studies. The area under the curve and integral of early morning salivary cortisol have been recommended to improve sensitivity and specificity of the biomarkers (Lu *et al.*, 2016, Zhang *et al.*, 2016). In an analysis of results of a 34-study meta-analysis and stress, difficulty comparing results and interpreting the data led to the proposal that the integral of salivary cortisol is used to reduce methodological variation and variability of results (Liu *et al.*, 2017).

Reduced feedback inhibition and glucocorticoid resistance are seen with early childhood trauma and the integral of the cortisol awakening response was used to show hyper-reactivity of the HPA axis in MDD (Lu *et al.*, 2016) . In contrast, the integral of the early morning awakening response over 90 minutes in young males was reduced when family conflict when measured by the family environment scale (Zhang *et al.*, 2016). Early morning salivary cortisol was found to be a valid screening biomarker for MDD (Owens *et al.*, 2014) and the biomarker appeared to differentiate depressive symptoms from anxiety (Nelemans *et al.*, 2014). The initial physiological response to stress, as seen in increased salivary cortisol, reduces over time (Miller *et al.*, 2007) and is affected by genotype where people with the 5HTTLPR L allele have a greater response than those with the SS allele (Ancelin *et al.*, 2017). Salivary cortisol is unaffected by age, weight, smoking, sleep duration, time of awakening or alcohol consumption (Pruessner *et al.*, 1997, Dmitrieva *et al.*, 2013).

Glucocorticoid Receptor (Nuclear Receptor Subfamily 3 Group C Member)

Nuclear receptor subfamily three group C member 1 (NR3C1) or Glucocorticoid Receptors (GR) are implicated in the aetiology of depression (Holsboer, 2000, Sapolsky, 2000) and are noted to be hyper-methylated in type II diabetes (Nontharat, 2015, Ouakinin, 2016). Hyper-methylation appears to be an epigenetic change which is a mediator of early childhood adversity related disorders (Tsankova *et al.*, 2007,

Covington *et al.*, 2009). Glucocorticoid resistance is maintained in part by pro-inflammatory biomarkers that reduce glucocorticoid receptor functionality (Pariante and Lightman, 2008b). Feedback responsiveness in the HPA system is improved from glucocorticoid resistance to normal feedback inhibition with antidepressant therapy (Pariante and Lightman, 2008b).

Combined Dexamethasone Suppression Test/Corticoliberin Stimulation Test

HPA axis dysregulation occurs in depressed patients exhibiting elevated cortisol, corticoliberin (CRH), impaired suppression of the dexamethasone suppression test, and a blunted adrenocorticotrophic hormone (ACTH) response to CRH (Aihara *et al.*, 2007, Schüle *et al.*, 2009). Evidence of HPA axis activation appears to have prognostic value and is associated with increased risk of depression relapse and even suicide (Varghese and Brown, 2001). In addition, hypercortisolism is linked to both insulin resistance and major depression (Weber *et al.*, 2000). Cortisol, the combined CRH/Dexamethasone Suppression Test (DST) test and the HPA axis have been reviewed by Young et al indicating potential as biomarkers in depression and to help reveal tipping transformations in HPA axis dysfunction (Young *et al.*, 2016).

Trier Social Stress Test (TSST) and CO_2 Stress Test

The TSST and CO_2 stress tests both activate cortisol and the human stress response with pre-response sensitivity to cortisol and post-stress resistance to cortisol (Kirschbaum *et al.*, 2008, Lu *et al.*, 2016). Early trauma also leads to increased activation of adult HPA axis and immune systems demonstrated with increased biomarkers TNFα, interleukin-6 (IL-6), IL-1β, and C-reactive protein (CRP) (Liu *et al.*, 2017).

B. Metabolic Dysfunction

Acute Phase Response

CRP has been shown to be elevated in depressed patients (Dowlati *et al.*, 2010). In the acute phase response Cortisol Binding Globulin (CBG) falls while CRP rises (Burtis *et al.*, 2015a). The ratio would increase sensitivity and demonstrate early development of an acute phase response making it one of the potential screening biomarker in depression.

Hypothalamo-Pituitary-Thyroid Axis (HPT Axis) and Autoimmunity

Thyroid stimulating hormone (TSH) fluctuates in a nyctohemeral rhythm (Sviridonova *et al.*, 2012) with a peak at 0200-0400 hours and trough at 1600-2000 hours (Persani *et al.*, 1995).

An increase in antithyroperoxidase antibodies (anti-TPO Abs) has been associated with MDD suggesting its use as a biomarker (Van de Ven *et al.*, 2012). A higher lifetime prevalence of depression was found in anti-TPO antibody positive versus negative individuals (Dufour, 2007). There is a reported association between anti-TPO Abs and lifetime diagnosis for major depressive disorder (Carta *et al.*, 2004b). Anti-TPO Abs are also associated with increased risk of hypothyroidism, a known causative factor in MDD (Marangell and Callahan, 1998, Carta *et al.*, 2004a, Fountoulakis *et al.*, 2004).

Low TSH in normal individuals appears to be linked to an increased rate of depression (Dayan and Panicker, 2009, Estrada *et al.*, 2014). Intracellular type 1 deiodinase determines thyroxine:tri-iodothyronine (T4:T3) ratio and phosphodiesterase determines TSH levels while both of these are connected to psychological well-being (Dayan and Panicker, 2009).

Hair T3, the long-term integral of plasma T3, the active hormone, has been shown to be significantly lower in depressed patients (Paus *et al.*, 2014, Wei *et al.*, 2014).

Leptin and Ghrelin

Ghrelin causes increased intracellular levels of reactive oxygen species (ROS) (Buldak RJ *et al.*, 2015) and is associated with oxidative stress and insulin resistance (Razzaghy-Azar *et al.*, 2016). In addition, women with interpersonal stress had higher ghrelin and lower leptin levels, suggesting the ratio would be more revealing than either biomarker concentration alone (Jaremka *et al.*, 2014). Leptin levels have also been shown to be significantly associated with low mood in normal weight women (Häfner *et al.*, 2012). Leptin and glucocorticoid receptors are co-located on hippocampal progenitor cells and act on neurogenesis through Glycogen synthase kinase (GSK-3) (Garza *et al.*, 2012).

Heart rate variability

Heart rate variability (HRV) is an established measure of cardiac vagal control (Rottenberg, 2007). Flickering reflects increasing asymmetry of fluctuations over time before a critical transition, which can theoretically be measured by assessing HRV. Flickering could be investigated using the R-wave interval time series in a 24 hour cardiac recording to predict underlying change in autonomic function. The low frequency component of RR-interval data has been used to analyse the stochastic change with persistence around a set-point, which relates to sympathetic and parasympathetic cardiac control (Zheng *et al.* (2013), and it is proposed that similar methodology could be used to detect a critical transition to MDD.

Carbohydrate metabolism and Haemoglobin A_{1c} (HbA_{1c})

Insulin resistance or impaired glucose tolerance, as indicated by the oral glucose tolerance test, fasting plasma glucose and $HbA_{1c,}$ have been linked to depression (Winokur *et al.*, 1988, Weber *et al.*, 2000, Chen *et al.*, 2010). HbA_{1c} is the area under the curve or integral of the average plasma glucose over the preceding 8-12 weeks, if red cell survival is normal. Approximately 50% of HbA_{1c} is derived from the plasma glucose over the preceding month and the remainder from the two months before that (Burtis *et al.*, 2015a). Increased variance is seen when the range of concentrations of glycated haemoglobin measured over time increases above baseline. It indicates that the plasma glucose concentration is, at times, elevated above the normal physiologically controlled range. These raised levels may not be detected by measuring random plasma glucose levels as they can be transitory. However, irreversible, non-enzymatic binding of glucose to the N-terminal valine amino acid on the beta globin chains on haemoglobin A occurs directly in proportion to the plasma glucose concentration. As it forms a stable ketoamine over the life of the red cell, the concentration of glycated HbA_{1c} detects those elevations, the increased variance in the plasma glucose and the area under the curve of plasma glucose concentrations over the life of that red cell.

Human leukocyte telomere length (LTL)

There is an association between major depressive disorder and telomere length ($p < 0.001$) and with severity of depression ($p = 0.03$) (Ridout *et al.*, 2016). Concurrent telomere length was more strongly associated than longitudinal telomere length suggesting more recent epigenetic change. The current hypothesis is that oxidative stress exposure and ageing lead to reduction in telomere length.

Intracellular Tipping Point Biomarkers

A Dysfunction of tryptophan metabolism in the serotonin/kynurenine/quinolinic acid pathways

Tryptophan Metabolism

Tryptophan is an essential amino acid and therefore supply is rate limiting if intake is inadequate. Tryptophan can only be transported across the blood brain barrier in its free form by a competitive and non-specific amino acid transporter (Chen and Guillemin, 2009). It then is incorporated into proteins or converted into serotonin or kynurenine. 99% of dietary tryptophan is metabolised down the kynurenine pathway (Mbongue *et al.*, 2015). Indolamine 2, 3-dioxygenase (IDO) is the first and rate-limiting enzyme of tryptophan metabolism through the kynurenine pathway. Alternatively, tryptophan can be metabolised in neurones to serotonin with the initial rate limiting enzyme tryptophan hydroxylase. Serotonin is metabolised to 5HIAA while kynurenine is metabolised to kynurenic acid, picolinic acid or quinolinic acid. In turn, quinolinic acid is converted to Nicotinamide Adenine Dinucleotide (NAD) and NAD phosphate (NADP) that is required for oxidative metabolism directly linking tryptophan supply to mitochondrial oxidative phosphorylation and intracellular energy production (Ruddick *et al.*, 2006). In states of increased consumption of tryptophan such as inflammation, production of serotonin is limited by necessity for protein synthesis and production of NAD and NADP, and this pathway is metabolically prioritised (Lundstrom *et al.*, 2005, Ruddick *et al.*, 2006). The ratio of kynurenine: tryptophan is an indicator for the activity of IDO (Mbongue *et al.*, 2015), which is an immunomodulatory enzyme (Ruddick *et al.*, 2006). Measurement of the differentials and integrals of kynurenine metabolites, including those as ratios to serotonin metabolites, can serve as biomarkers for activity of these related, but competing, pathways.

Leucocyte 5HT $_{1A}$ receptors

In depressed patients, 5-HT_{1A} receptor expression on leucocytes is inversely correlated with serotonin activity in plasma and 5-hydroxyindoleacetic acid (5-HIAA) concentration in urine (the end-product of serotonin metabolism). This occurs through a negative feedback mechanism and affects the HPA axis (Zhang *et al.*, 2014).

Resistance to change is the second derivative differential, where the rate of change in concentration of a biomarker is measured. Practically, that would require multiple measurements, which can be clinically untenable. However, measuring the white cell response to the alterations in the rate of change, in this case resistance to negative feedback inhibition and down-regulation of receptor expression, is a biomarker reflecting the effect of changes in the velocity of $5HT_{1A}$ concentration pathophysiologically.

Galanin

Galanin is found in gut and brain tissue. It is an inhibitory neurotransmitter co-located with serotonin and noradrenaline, with the highest concentrations in the CNS found in the hippocampus where it acts as an inhibitory neuromodulator modifying glutamate, but not GABA transmission. It has been linked to depression and anxiety through modulation of monoamine transmitters and the neuroendocrine system (Kozlovsky *et al.*, 2009) (Lundstrom *et al.*, 2005). Galanin also stimulates the HPA axis increasing CRH and ACTH release and modulating the HPA axis (Tortorella *et al.*, 2007). In addition, there are Galanin receptors in the adrenal medulla, so it is involved in regulation of the autonomic nervous system (Tortorella *et al.*, 2007).

B Inflammation and Mitochondrial Oxidative Stress

Oxidative stress has been implicated in the pathophysiology of MDD and can be reversed by serotonin reuptake inhibitors (Bilici *et al.*, 2001, Khanzode *et al.*, 2003).

The free radical theory proposes that ROS produce mitochondrial damage, which in turn reduces energy supply through impaired oxidative phosphorylation. Damaged proteins then activate mechanistic target of rapamycin (mTOR) for autophagy, further potentiating long term cellular senescence (Kriete *et al.*, 2010).

Tumour Necrosis Factor (TNFα)

TNFα has been proposed as a biomarker in MDD reflecting HPA axis dysfunction (Tanabe and Nomura, 2007, Berthold-Loslben and Himmmerich, 2008). It has also been associated with activation of the cytokine system and the behavioural changes associated with major depressive disorder and sickness behaviour, including malaise, pyrexia, social isolation, anhedonia, anorexia, loss of concentration and lethargy (Reichenberg *et al.*, 2001). Cytokine activation leads to activation of serotonin transporters and relative serotonin deficiency (Zhu *et al.*, 2006). This is mediated by TNFα and stimulation of IDO with resultant depletion of tryptophan (Wichers and Maes, 2002). TNFα blockers used in the treatment of psoriasis are associated with more than 50% improvement in Hamilton Depression Scale, Beck Depression Inventory and Chronic Illness Therapy Fatigue Scale, and with reduction in sickness behaviour (Wichers and Maes, 2002). Obesity increases TNFα over time leading to sickness behaviours similar to those seen in MDD (Ouakinin, 2016).

NF-κB

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that controls transcription of deoxyribonucleic acid (DNA), cytokine production and cell survival (Lawrence, 2009, Tornatore *et al.*, 2012), and NF-κB has also been implicated in synaptic plasticity and memory (Albensi and Mattson, 2000, Meffert *et al.*, 2003). NF-κB is a first responder to harmful cellular stimuli (Vlahopoulos *et al.*, 2015). Known inducers of NF-κB activity are highly variable and

include ROS and TNFα (Chandel *et al.*, 2000). Fidelity of feedback responses between diverse cell types and the immune system depends on the integrity of mechanisms that limit the range of genes activated by NF-κB, allowing sole expression of genes which contribute to an effective immune response and subsequently, a complete restoration of tissue function after resolution of inflammation (Vidal *et al.*, 2012). It is understood that chronic low-grade inflammation plays a key role in the initiation, propagation, and development of metabolic diseases consistent with its central role in coordinating inflammatory responses, numerous recent studies have implicated the transcription factor NF-kB in the development of such diseases (Baker *et al.*, 2011). Several studies during the past two decades have highlighted the key role of the inhibitor of kappa B kinase (IKK)/NF-κB pathway (Israël, 2010) in the induction and maintenance of the state of inflammation that underlies metabolic diseases such as obesity and type 2 diabetes (Hayden and Ghosh, 2008).

Zinc and Magnesium

Both Zn and Mg are known to inhibit the NMDA glutamate receptor complex and improve mood in depression (Barragan-Rodriguez *et al.*, 2008, Sawada and Yokoi, 2010) and could form part of a profile of biomarkers in screening for MDD.

Brain Derived Neurotrophic Factor (BDNF)

BDNF is critically involved in neuroplasticity in the neural networks giving rise to sickness behaviours and behaviours in depression (Schinder and Poo, 2000, Pittenger and Duman, 2008). BDNF signals through glycogen synthase kinase 3 (GSK-3) (Ricken *et al.*, 2013). BDNF levels are noted to be reduced in the serum of depressed people, and increase in response to antidepressant therapy (Sen *et al.*, 2008, Molendijk *et al.*, 2014). Higher BDNF levels were also associated with better treatment outcomes.

Glycogen Synthase Kinase 3 (GSK-3)

GSK-3 is protein kinase and active in a number of central intracellular signalling pathways, including cellular proliferation, migration, glucose regulation, and apoptosis. Due to its importance across numerous cellular functions, GSK-3 activity is subject to tight regulation (Cole and Sutherland, 2008). The activity of GSK-3 is far greater in the nucleus and mitochondria than in the cytosol in cortical neurons (Bijur and Jope, 2003). Lithium is an inhibitor of GSK-3 and is an effective mood stabiliser in Bipolar Disorder, and as an augmenting agent in treatment refractory MDD (Taylor *et al.*, 2015). Other GSK-3 inhibitors show promise in the treatment of type 2 Diabetes Mellitus, which is, in itself, correlated with depression (Rayasam *et al.*, 2009). Cole and Sutherland have reviewed available methods and concluded that the challenge with GSK-3 measurements is that different methods give significantly disparate results (Cole and Sutherland, 2008).

Circular RNAs

Circular, covalently linked segments of RNA in plasma have been identified as having utility as novel biomarkers in neurological disorders and depression (Cui *et al.*, 2016, Ma *et al.*, 2016).

Mechanistic Target of Rapamycin (mTOR)

MTOR is a protein kinase that regulates cell growth, energy production, cell survival and autophagy. It is a central intracellular regulator of energy production, oxygen levels and nutrient use (Tokunaga *et al.*, 2004). It is implicated in MDD through control of cellular metabolism (Tokunaga *et al.*, 2004), and ketamine, an N-Methyl-D-Aspartic acid (NDMA) antagonist and its metabolite Nor-Hydroxy-Ketamine (NHK), an Amino-hydroxy-Methyl-isoxazole-Propanoic Acid (AMPA) agonist, (Zanos *et al.*, 2016, Machado-Vieira *et al.*, 2017), may act as antidepressants through rapid activation of the

mTOR pathway, increasing spine formation and synaptic transmission in the prefrontal cortex (Welberg, 2010) (Li *et al.*, 2010). Dysfunctional activity of mTOR has been associated with reduced synaptic plasticity and impaired learning and memory (Oddo, 2012), one of the six cognitive domains impaired in MDD revealed as difficulty with decision making and learning new tasks (American Psychiatric Association, 2013). Reduced levels of mTOR are associated with increased cell lifespan, improved glycolysis and decreased removal of dysfunctional intracellular components (Lang and Borgwardt, 2013).

Interleukin 6 (IL-6) and IL-10

Repeated IL6:IL10 may show a change to a critical transition by revealing a change to a pro-inflammatory state to through positive autocorrelation. IL-10 is one of the anti-inflammatory family of cytokines (Oral *et al.*, 2006) in contrast to IL-6 which is pro-inflammatory cytokine (Heinrich *et al.*, 2003) and anti-inflammatory myokine (Pedersen and Febbraio, 2008) and these interleukins have a role in modulation of neuronal plasticity (Vidal *et al.*, 2012).

Sickness behaviour has been shown to be mediated by pro-inflammatory cytokines (Dantzer, 2009). IL-6 and TNFα have been shown to be elevated in a meta-analysis of studies on depressed patients (Dowlati *et al.*, 2010). Cytokines have been also found to be related to low mood in males (Chen *et al.*, 2010).

Measuring IL6 and IL10 levels multiple times can show a change from a balanced inflammatory state and normal predominance of anti-inflammatory activity, to a pro-inflammatory state with pro-inflammatory biomarkers elevated. A ratio can detect this change earlier improving sensitivity, which is an advantage for a screening biomarker.

Salivary cortisol and IL-6 were linked to MDD in an 8 study meta-analysis of patients on interferon alpha (IFN-α) therapy (Machado *et al.*, 2017).

Discussion

The four functions of biomarkers (screening, diagnosis, prognostic and monitoring) have inherently differing characteristics. By focussing on those for screening, and applying CBP theories and Scheffer et al's five generic categories of tipping point transformations that occur before catastrophic bifurcations, four groups of biomarkers, and the integrals and differentials of those, were identified. They were identified and divided into extracellular and intracellular tipping point biomarkers:

Extracellularly - HPA axis and metabolic dysfunction

Intracellularly - dysfunction of tryptophan metabolism, and inflammation and mitochondrial oxidative stress.

Potential screening biomarkers were proposed, serving as surrogates for the tipping points as listed in Tables 1 & 2. It is the combination of a group of biomarkers that has the potential to lead to a screening profile to aid clinicians in screening for MDD in high risk patients.

The approach used here can also be applied to diagnostic, prognostic and monitoring biomarkers but will, in all probability, result in a differing profile of biomarkers for each function.

Conclusion

Further biomarker research is reliant on research into the physiology and pathology of MDD. It is proposed that the next steps in the research of biomarkers in MDD are to develop and test a comprehensive list of target biomarkers for the tipping points in

MDD by identifying relevant system specific biomarkers that fulfil the essential characteristics for screening, as opposed to diagnosis, prognosis or monitoring response to therapy. The use of the theoretical approach and mathematical framework to identify biomarker changes in the tipping points prior to catastrophic bifurcations will potentially aid in screening, diagnosis, prognosis and monitoring of MDD.

Declaration of Interests

The authors report no conflicts of interest.

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Table 1: Proposed extracellular biomarkers in screening for MDD

Extracellular Tipping Point Biomarkers	
Serum unless otherwise stated	
HPA Dysfunction and Glucocorticoid Resistance	
Skew	Salivary cortisol on awakening and at 60 minutes
Resistance to change	Salivary cortisol in response to awakening at baseline, 30, 60, 90 minutes
	Nuclear receptor subfamily 3 group C member 1 (NR3C1 or glucocorticoid receptor) concentration in leucocytes
	Combined Dexamethasone Suppression Test (DST) and Corticoliberin (CRH) stimulation test
	Trier Social Stress Test (TSST) stress test using salivary cortisol concentration and corticotrophin (ACTH) at 10, 30 and 60 minutes or CO2 stress test using same parameters as TSST
Flickering	No biomarker identified
Increased variance	24 hour urine free cortisol or early-morning urine free cortisol to creatinine ratio
Autocorrelation	Repeated methylated nuclear receptor subfamily 3 group C member 1 (NR3C1 or glucocorticoid receptor) concentration in leucocytes
Metabolic Dysfunction	
Skew	C-reactive protein: cortisol binding globulin (CRP:CBG) ratio
	Anti-thyroperoxidase antibody concentration (anti-TPO antibodies)
	Ratio of leptin to ghrelin concentrations
Resistance to change	Peak thyroid stimulating hormone (TSH) concentration at 0300 hours
	CRP:CBG ratio with multiple tests over time
Flickering	Heart rate variability
Increased variance	Haemoglobin A_{1C} concentration
Autocorrelation	Hair free T3 concentration
	Repeated Haemoglobin A_{1C} over time

Table 2: Proposed intracellular biomarkers in screening for MDD

Intracellular Tipping Point Biomarkers	
Serum unless otherwise stated	
Dysfunction of Serotonin and Kynurenine Pathways	
Skew	Ratio of concentration of picolinic acid to kynurenic acid
	Ratio of concentration of quinolinic acid to kynurenic acid
Resistance to change	5 hydroxytryptamine 1A ($5\text{-}HT_{1A}$) receptors in leucocytes
Flickering	No biomarker identified
Increased variance	Ratio of 24 hour urine picolinic acid to 5-hydroxy indole acetic acid (5HIAA)
	Ratio of 24 hour urine quinolinic acid to 5-hydroxy indole acetic acid (5HIAA)
Autocorrelation	Tryptophan to kynurenine; tryptophan to quinolinic acid; tryptophan to free serotonin (5-HT) ratios
Inflammation and Mitochondrial Oxidative Stress	
Skew	Galanin concentration
	Tumour necrosis factor alpha (TNFα) concentration
	Nuclear factor kappa-B (NF-κB) concentration
	Zn and Mg concentrations
	Brain derived neurotrophic factor (BDNF)
	Circular RNAs (circRNA)
Resistance to change	Concurrent telomere length in leucocytes (LTL)
	Glycogen synthase kinase 3 (GSK-3) activity in leucocytes
Flickering	Heart rate variability
Increased variance	24 hour urine ratio kappa to lambda light chains for NF-κB or early-morning kappa
	light chain to creatinine ratio in urine for NF-κB
	Concentration of circular RNAs (circRNAs)
Autocorrelation	Mechanistic target of rapamycin (mTOR) activity in leucocytes
	Ratio of interleukin 6 to interleukin 10 (IL-6: IL-10)